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Conformational Changes in the Lac Repressor Protein Effect DNA Loop Energetics and Topology

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DNA looping mediated by the Lac repressor protein is a classic exemplar for understanding protein-DNA interactions and allosteric mechanisms in gene regulation. When the repressor protein binds to two distant operator sites on substrate DNA, it causes the formation of DNA loops. Most of the previous study of this system has focused on either the properties of the DNA loop created by the protein-DNA interaction or seeks to explain the possible conformational changes of the protein itself. Here we take a combined approach by considering the effects of protein deformation on the topology and energetics of the induced looped-DNA. We begin by creating a collection of 3D atomic coordinate models of the protein to simulate the structural fluctuation consistent with earlier studies. By incrementally rotating segments of the protein about likely sites of flexibility these new models provide a large distribution of constraint parameters for the generation of looped-DNA coordinates. We then employ a novel approach to obtain minimum energy looped structure whereby the potential energy of elastic deformation is optimized. In this way we are able to describe energy landscapes that provide clues to how variations in protein structure contribute to DNA loop stability and topology.

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Determination of Free Energy Profiles for Polynucleotides Translocation through Mutant α-Hemolysin Nanopores

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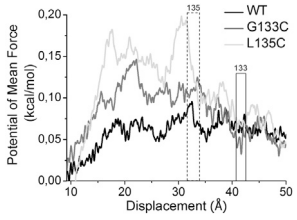
The understanding of nucleotides' interaction with the Staphilococcus aureus α-hemolysin nanopore is of special interest because some studies suggest several methods for using this nanopore incorpored in a planar lipid bilayer as a real time DNA sequencer. The main operational difficulty to obtain a DNA sequencer is the high DNA translocation speed through the nanopore, which hinders proper base discrimination. Strategies for producing increasing the residence time of the DNA and the interaction energy with the nanopore include site directed mutations and chemical functionalization. In this work we used nonequilibrium molecular dynamics simulations and Jarzynsnki's relation (JR) to obtain the free energy profiles of DNA translocation through α-hemolysin nanopores, in its native form or mutated by cysteine in strategic positions previously selected by molecular modeling investigations: G133 and L135. Compared to the native form, there was a considerable increase in the minimum energy needed for DNA transport; we also observe, in the point of mutation, a PMF increase in L135C and decrease in G133C. We intend use the obtained information about the interactions of DNA-nanopore to theoretically predict promising nanopores to be tested experimentally.



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Direct Single-Molecule FRET Imaging of the Eukaryotic Initiation Factor 4A Reveals Large Conformational Transitions During RNA Unwinding

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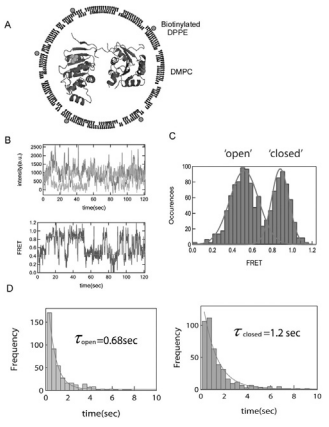
The translation initiation factor eIF4A is the prototypical DEAD-box RNA helicase and it has a ''dumbbell'' structure consisting of two domains connected by a linker. Our previous studies have shown that eIF4AI/eIF4H complex can bind directly to loop structures and repetitively unwind the RNA hairpin (1). Here, we report the direct and real-time observation of the conformational change of double labeled eIF4A during RNA unwinding using single-molecule FRET. We demonstrate that the eIF4A in the presence of eIF4H can repetitively unwind the RNA hairpin substrate by undergoing large 'open' and 'closed' conformation using the energy from ATP hydrolysis. Our experiments directly track the conformational changes in the catalytic cycle of eIF4A and eIF4H in real time and this correlate precisely with the kinetics of RNA unwinding. We find that the small molecule, hippuristanol, can inhibit the helicase activity of eIF4A/eIF4H, by locking eIF4A at its closed conformation, thus disabling its ability to hydrolyze ATP.



1. Y. Sun et al., Nucleic Acids Res 40, 6199 (Jul, 2012).

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Role of Dead Box Helicases in HIV-1 Rev Function: a Single-Molecule Approach

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The HIV-1 Rev (Regulator of Expression of Virion) protein activates nuclear export of unspliced and partially spliced viral mRNAs, which encode the viral genome and the genes encoding viral structural proteins. Rev interacts with a highly conserved region, the Rev Response Element (RRE), located within the viral mRNA. Initially, a single Rev monomer binds to stem loop IIB of the RRE, whereupon additional Rev monomers are recruited to the RRE through a combination of RNA-protein and protein-protein interactions, resulting in the formation of a functional nuclear export complex. In addition, several cellular host proteins, such as the DEAD box helicases DDX1 and DDX21 are known to be required for efficient Rev function in vivo, although their precise role is unknown. In this study, a variety of single-molecule fluorescence spectroscopic methods were used to dissect the role of DDX1 and DDX21 during assembly of Rev-RRE complexes. To facilitate these studies, the large DDX1 and DDX21 proteins were enzymatically labeled with bright and photostable dyes, while Rev was labeled at a single cysteine by maleimide chemistry. Single-color TIRF measurements with labeled Rev were used to monitor individual Rev monomer binding steps during oligomeric Rev-RRE assembly. Moreover, single-color experiments with labeled DDX1 or DDX21 reveal that each helicase is capable of binding directly to the RRE. Two-color colocalization and FRET measurements were used to monitor the simultaneous binding of both Rev and DDX1 to the RRE, revealing the temporal correlation between individual protein binding steps. Together, these studies are revealing how DEAD box helicases are able to promote the oligomeric assembly of Rev on the RRE, thereby acting as cellular cofactors of HIV-1. Supported by NIH P50 grant GM082545.

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DEAD Box Helicases in Rnp Granule

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Processing body (p-body) granule is located at the cytoplasm of eukaryotic cells and serves as a molecular hub for collection of mRNA and RNA processing enzymes. (Science 324 pg. 26 June 2009) P-body has been implicated for mRNA storage, splicing, degradation and translational repression, but how it assembles and carries out the diverse array of functions is not clearly understood. Among RNA processing enzymes, RNA helicases are thought to play the central role by active ATP hydrolysis, contributing to the liquid-like property of P-body granules.

In this study, a DEAD-box helicase in p-body granules is characterized by single molecule FRET analysis. One of RNA helicases from C.Elegans, Laf-1 is composed of an RGG boxes at its N-terminus and polyglutamine tract at its C-terminus with DEAD box helicase core in its center. Laf-1 binds exclusively to single strand RNA (ssRNA) and shows a weak ATP hydrolysis activity stimulated by single stranded RNA. Upon binding ssRNA, Laf-1 initially wraps/bends ssRNA and exhibit dynamic wrapping-unwrapping interaction with the RNA. Furthermore, Laf-1 promotes RNA annealing 10 to 100 fold faster, but does not show any unwinding activity. Its ability to wrap/unwrap ssRNA and anneal complementary ssRNAs may explain the role of Laf-1 in assembling the p-body granules and maintaining dynamic, liquid-like property.

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RNA Helicases on the Move

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We have investigated the mechanical properties of the RNA-helicase-motor proteins in single-molecule assays using Magnetic Tweezers. RNA helicases